

L8 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2000 ACS  
TI Ionization and tautomerism of **fluorescein**, **rhodamine**  
B, N,N-diethylrhodol and related dyes in mixed and nonaqueous solvents  
AU Mchedlov-Petrusyan, Nikolay O.; Kuchtik, Valentina I.; Alekseeva, Vera I.  
SO Dyes Pigm. (1994), 24(1), 11-15  
CODEN: DYPYDZ; ISSN: 0143-7208  
AB The protolytic equilibria of **fluorescein**, **rhodamine** B and  
of the aryl amino-oxyanthene dye, N,N-diethylrhodol (a 'hybrid' of  
**rhodamine** B and **fluorescein**) were studied in aq. DMSO  
and EtOH (9:1 org. cosolvent). The pKa values of these dyes, as  
well as of related substances, were detd. On the basis of the visible  
absorption spectra in various solvents conclusions were made about  
tautomerism in the dye mols. Values of the tautomeric equil. consts. and  
of the microscopic ionization consts. were obtained. Some new data on the  
tautomerism of oxyanthene monoanions in MeOH were presented.

=> d t f a n s w e r

L8 ANSWER 3 OF 4 MEDLINE  
TI Phagosomal pH determination by dual fluorescence flow cytometry.  
AU Vergne I; Constant D; Lianesle G  
SO ANALYTICAL BIOCHEMISTRY, (1998 Jan 1) 255 (1) 127-35.  
Journal Code: 4NKL ISSN: 0003-2697.  
AB Several methods have been developed to measure the pH of phagosomes, using  
**fluorescein** derivatives as reporter of pH, and spectrofluorimetry,  
fluorescence microscopy, or flow cytometry as quantification technique.  
All have major disadvantages, including either a slow or inaccurate  
response. In the present study, pH determination was achieved on J774-cell  
phagosomes containing dual-labeled zymosan particles using dual  
fluorescence flow cytometry with an argon-ion laser excitation wavelength  
at 488 nm. This allowed zymosan-containing macrophages to be distinguished  
from other cells and their fluorescence to be measured rapidly. The use of  
a new probe, namely Oregon Green 488 which has a pKa lower than  
carboxyfluorescein with the same maximum excitation and emission  
wavelengths, allowed investigation of pH value below 5. The dual labeling  
with Oregon Green 488 and carboxyterramethylrhodamine as pH-sensitive and  
pH-insensitive probes, respectively, overcame the absence of an isobestic  
point in the Oregon Green 488 spectrum. The phagosomal pH was determined  
using a calibration curve of phagosomal pH established by adding  
ionophores to phagocyte suspension and measuring the fluorescence  
intensity ratio (535 nm/585 nm) for different pHs. A phagosomal pH of 4.5  
± 0.1 can be accurately determined. This method permits pH measurements  
down to 4, even in the presence of nonengulfed reporter particles.

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